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Review

Senescence and tissue fibrosis: opportunities for therapeutic targeting

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Cellular senescence is a key hallmark of aging. It has now emerged as a key mediator in normal tissue turnover and is associated with a variety of age-related diseases, including organ-specific fibrosis and systemic sclerosis (SSc). This review discusses the recent evidence of the role of senescence in tissue fibrosis, with an emphasis on SSc, a systemic autoimmune rheumatic disease. We discuss the physiological role of these cells, their role in fibrosis, and that targeting these cells specifically could be a new therapeutic avenue in fibrotic disease. We argue that targeting senescent cells, with senolytics or senomorphs, is a viable therapeutic target in fibrotic diseases which remain largely intractable.

Highlights

Cellular senescence is present in tissue fibrosis across multiple organs.

In animal models of fibrosis, the selective elimination of senescent cells, pharmacologically or genetically, retarded the fibrosis.

Targeting senescence for clinical gain using so-called senolytics is currently ongoing in fibrotic diseases.

Cellular senescence

Cellular senescence (see Glossary), one of the hallmarks of aging, has emerged as a process with fundamental roles in physiological processes, age-related illnesses, and a variety of other pathologies [1]. The phenomenon was first described in the 1960s [2,3] when researchers noted that cultured cells ceased proliferation after a certain number of *in vitro* passages, a phenomenon they attributed to chronological ageing. Importantly, it was also noted that tumor cells failed to undergo this cell cycle arrest, giving rise to the notion that cellular senescence was a mechanism to suppress the development of cancer [4] (Box 1), while senescence can also occur in post-mitotic cells such as neurons.

Cellular senescence appears to be linked with tissue fibrosis in a variety of age-associated and age-independent conditions. Idiopathic pulmonary fibrosis (IPF) (Box 2), an age-related disease, is associated with enhanced markers of senescence [5]. SSc is an autoimmune connective tissue disease whose hallmarks are inflammation and skin and lung fibrosis. SSc also has hallmarks of senescence (Box 2). These data add to the growing body of literature implicating senescence in fibrosis. In this review, we aim to appraise the literature regarding senescence and fibrosis, identify gaps in our knowledge, and appraise the possibility of therapeutic targeting of cellular senescence, or senotherapy. This is timely, as senescence has been linked to fibrosis, and targeting senescence is now a real possibility.

Defining senescence: aging versus premature senescence

Chronological ageing can be defined as the time-related deterioration of physiological functions necessary for survival and fertility. Defining senescence is somewhat more difficult. We will use the definition that 'senescence is a cellular program that induces a stable growth arrest accompanied by distinct phenotypic alterations'. These phenotypic changes include metabolic reprogramming, telomere dysfunction, histone modifications, altered autophagy, expression of characteristic cell surface markers, and acquisition of the senescence-associated secretory phenotype (SASP) [6]. The purpose of the growth arrest, the hallmark of cellular senescence, is a cell-autonomous anticancer mechanism limiting malignancy. Key alterations in metabolism include alterations in mitochondria mass and reactive oxygen species (ROS) production,

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Box 1. Cellular senescence

It is now appreciated that cellular senescence is characterized by sustained proliferative arrest, resistance to apoptosis, metabolic alterations, and increased generation of reactive oxygen species (ROS), along with the acquisition of a senescence-associated secretory phenotype (SASP) [107,108]. The SASP underlies chronic inflammation in aging (inflammaging) and can initiate or exacerbate a variety of pathological conditions. The process of cellular senescence has gained increasing interest, partly due to increased lifespan and the increased incidence of age-related diseases [109].

disruption of autophagy, and disruption of lipid metabolism, with large increases in ceramide and sphingosine.

Ageing is a phenomenon that all animals share. While senescence is associated with ageing, there are instances where there may be 'premature senescence' uncoupled from chronological ageing. An extreme example of this is progeria, also known as Hutchinson–Gilford syndrome, where children demonstrate features of ageing. Progeria is an extremely rare genetic syndrome caused by a mutation in lamin A, resulting in a mutated form that is permanently farnesylated, named progerin [7,8]. This leads to altered hydrophobicity and increased nuclear membrane stiffness, ultimately leading to changes in histones, changes in epigenetic marks, DNA damage, and senescence [9].

While this is an extreme example of a premature senescence phenotype, it is likely that premature senescence in other diseases is occurring uncoupled from ageing. Indeed IPF for example, has been proposed to be a disease of accelerated ageing [10]. Mutations in telomerase (the enzyme responsible for adding DNA to telomeres) are associated with IPF and, in SSc, autoantibodies targeting telomers have recently been identified [11]. This indicates that in SSc, accelerated ageing could occur via telomerase inhibition mediated through antibodies targeting these components. Such autoantibodies would target all cell types too. Furthermore, mice lacking telomerase or with a telomere dysfunction in type II alveolar cells, develop an IPF-like disease [12], indicating its critical importance.

Senescence and SASP: a potent protein milieu

Senescence is associated with a variety of phenotypic alterations including increased cellular size [13]. Although a variety of markers have been associated with senescence, no single marker

Box 2. Senescence in idiopathic pulmonary fibrosis and systemic sclerosis

It has been shown that targeting senescent cells improves lung fibrosis and overall health in a preclinical animal model of idiopathic pulmonary fibrosis (IPF) [43]. Multiple markers of cellular senescence have been shown to be elevated in fibrotic lungs. In addition to the cell cycle inhibitor p16 [110], IPF cells display positive SA-beta galactosidase staining, a marker of cellular senescence, along with altered replicative capacity [111]. Additional preclinical lung fibrosis models have also demonstrated features of senescence [48,49,112–115]. Most importantly, the elimination of senescent cells in these models using either genetic or pharmacological strategies, has shown amelioration of lung fibrosis [43,99,116]. These observations suggest that cellular senescence is critically involved in lung fibrosis.

Systemic sclerosis (SSc) is a chronic autoimmune disease associated with microvascular abnormalities, inflammation, and, most importantly, fibrosis affecting the skin, lungs, and many other organs [117,118]. Recent studies have provided evidence of the potential role of cellular senescence in SSc. One study demonstrated that mitochondrial ROS-induced senescence paralleled proinflammatory and fibrotic markers in SSc fibroblasts [76]. Furthermore, NADPH oxidases (NOX) functioned as profibrotic stimuli feeding a forward loop and reinforcing fibrosis [119]. Shortening of telomeres, a hallmark of aging and a trigger for inducing cellular senescence, has also been noted in SSc. In particular, lymphocytes were shown to have reduced telomere length compared with age-matched controls, particularly in SSc patients with significant interstitial lung disease [81,120]. Importantly, recent studies have described the accumulation of senescent cells in SSc. Notably, skin biopsies from SSc patients demonstrated upregulation of gene signatures indicative of cellular senescence [77]. Similarly, lung biopsies from patients with SSc-associated interstitial lung disease (ILD) showed increased senescence signatures [81]. IPF is characterized by fibrosis of the lungs with no clear etiology. The disease primarily affects those over 65 years of age and is associated with both advancing age and cellular senescence. In many cases of this disease, there are alterations in telomeres.

Glossary

Cellular senescence: can be defined as sustained or irreversible cell cycle arrest, associated with acquisition of the senescence-associated secretory phenotype (SASP), altered metabolism, and expression of cell surface markers that allow recognition by the immune system. Accumulation of senescent cells can be transient or chronic, and homeostatic or pathological. Virtually all cells can enter cellular senescence under stressful conditions.

Modified Rodnan skin score (MRSS): a clinical tool to quantify the extent of skin fibrosis in systemic sclerosis. It is taken from 17 different areas of skin on the body and ranges in score from 0 to 51; higher scores indicate an increased severity of fibrosis.

Proteostasis: protein homeostasis or 'proteostasis' is the process that regulates proteins within the cell to maintain the health of both the cellular proteome and the organism.

Senolytics: drugs that preferentially induce apoptosis in senescent cells.

Senomorphics: also termed senostatics or SASP inhibitors, modulate SASP selectively by blocking inflammatory paracrine signalling from cells undergoing senescence.

Stimulator of interferon genes (STING): an intracellular innate immune receptor for intracellular DNA, which evolved to sense free DNA within the cell. When free DNA is sensed cGAS generates the second messenger cGAMP that activates STING.

unequivocally identifies it. Because the main attribute of cell senescence is irreversible cell cycle arrest, cycle markers such as p16, alternative reading frames (ARFs), and p21 are often upregulated in senescence, with p16 and ARFs being induced by mitogenic stress [14]. Since the DNA damage response is initiated in senescent cells, derived from a variety of different types of senescent stimuli, phosphorylated histone 2AX (H2AX) is often commonly associated with senescent cells [15]. Furthermore, telomeres at the ends of chromosomes are often reduced in length and are the target of DNA-damaging agents and stress-inducing stimuli [16].

Mitochondrial dysfunction and decreased mitophagy are also described as hallmarks of senescence. Mitophagy is a specialized form of autophagy, where damaged or dysfunctional mitochondria are degraded and recycled using the autophagy cellular machinery. This is a continuous cycle, and impaired mitophagy has been demonstrated in cellular senescence [17]. This has been associated with increased mitochondrial mass and ROS, and with cigarette-induced senescence [18]. Mechanistically cytosolic senescent p53 binds parkin and disturbs its translocation to damaged mitochondria and thus reduces mitophagy [19]. It was further demonstrated that stress-induced senescence is associated with reduced autophagy [20] while restoring autophagy protects cells from oxidative stress-induced senescence [21].

The SASP as a key feature of senescence

Although multiple features are associated with senescence, as described earlier, one of the most widely understood is the SASP. The SASP is a surfeit of inflammatory mediators that are synthesized and released from senescent cells [22]. These include cytokines, chemokines, proteases, growth factors, and lipids. Unfortunately, the precise composition of the SASP is variable. It is important to note that the stimulus used to provoke senescence and the complex time-dependency in culture (i.e., when you measure it) will determine the composition of the SASP. Although there is some variability in the composition of the SASP, it is generally accepted that the main constituents are proinflammatory factors that include interleukin (IL)-1β, IL-6, IL-8, transforming growth factor (TGF)-β, the matrix metalloproteases 1 and 3 (MMP1 and MMP3), and some chemokines [23,24].

What is the role of the SASP? This is a double-edged sword. On the one hand the SASP is detrimental and is associated with aging disease. It was demonstrated that Janus kinase inhibitors retarded SASP release from senescent adipocytes that mitigated age-related frailty [25], and inducible specific deletion of senescent cells *in vivo* attenuated age-related disease in an animal model [26], thus demonstrating that the SASP is directly pathogenic. In addition, the SASP containing IL-8 mediated enhanced metastasis of stromal cancer cells [27]. On the other hand, it has been shown that SASP factors can initiate the innate and adaptive immune response to tumours to promote tumor clearance [28]. Furthermore, the SASP has been found to promote tumor clearance via natural killer cells, which was abrogated by blocking CCL2 [29], and it has recently been noted that in p53-mediated senescence, the SASP can impair chemotherapy and clinical outcomes [30]. Considering all these aspects, it is clear that the role of the SASP is cell-specific and context-specific.

SASP regulation

SASP regulation occurs on multiple levels, including chromatin regulation and transcription. It was recently shown that the H3-specific demethylase KDM4 was critical for the SASP [31]. KDM4 is the enzyme that mediates the demethylation of H3K9/H3K36 of chromatin, leading to permissive chromatin leading to SASP generation. Interestingly, a recent study on oncogene-induced senescence identified that there was an increase in the active histone markers H3K79me2/3 in the chromatin of senescent cells [32]. It was found that this is critical for the SASP and not cell cycle arrest, and that the methyltransferase disruptor of telomeric silencing 1-like (DOTL1) that

regulates this epigenetic mark is critical in mediating the oncogene-induced SASP [32]. Interestingly, it was also shown that intracellular chromatin release leads to activation of the SASP through **stimulator of interferon genes** (**STING**) activation after the stimulation of cyclic GMP–AMP synthase (cGAS) and cyclic guanosine monophosphate–adenosine monophosphate (cGAMP) [33]. It was also shown that DOTL1 regulation in the previous study was downstream of STING [32]. Upstream regulators of SASP include the transcription factor NF-kB. It has also been demonstrated that the stress-inducible kinase P38 mitogen-activated protein kinase (MAPK) is a key regulator of the SASP and is independent of the DNA damage response [34,35]. The transcription factor GATA-binding protein 4 (GATA4) is also a key regulator of the SASP. Normally, GATA4 is degraded by autophagy but, in response to senescence-inducing stimuli, the autophagic degradation of GATA4 is repressed and this drives the SASP via ataxia-telangiectasia mutated (ATM). Zinc finger and scan domain containing 4 (Zscan4) has been identified as a key mediator of the SASP, and genetic depletion of Zscan4 significantly reduces SASP release after DNA damage [36]. The different regulators of the SASP likely reflect the actual inducer of senescence and the fact that the SASP appears at different time points in different cell types.

Senescence in tissue fibrosis

Acute or transient cellular senescence has homeostatic functions. In particular, the SASP can induce an immune response upon infection and removal of senescent cells, as well as orchestrate the remodeling and regeneration of tissue during development or wound repair. By contrast, chronic senescence, with a persistent accumulation of senescent cells, together with the proinflammatory and profibrotic presence of the SASP, contributes to development and progression of diseases. Indeed, persistent senescence has been implicated in various conditions, including fibrosis [37]. Specifically, cellular senescence has been implicated in kidney fibrosis, cardiac fibrosis, liver fibrosis, and lung fibrosis, to name a few [38–41]. Depletion of senescent cells in transgenic mouse models or by pharmacological approaches resulted in a decrease in lung fibrosis [42,43]. In the kidney, pharmacological depletion of senescent cells in mice resulted in improved kidney function and reduced fibrosis [44]. Similarly, an association between cellular senescence and resultant liver fibrosis was shown [45]. However, selective removal of senescent cells in a mouse model or a p16 and p21 double knockout mouse, in which a wound healing model was used, led to impaired wound healing in the early phase and was partially dependent on platelet-derived growth factor (PDGF)-A [46]. It has also been demonstrated that senescence is induced by wound healing to curb fibrosis through the matricellular protein cellular communication network factor 1 (CCN1). Mice that express a CCN1 mutant with a senescence-defective mutation had fewer senescent cells and less fibrosis than wild-type mice [47]. The profibrotic or regenerative role of senescence may reflect chronicity. In other words, early senescence induction is positively beneficial in restoring tissue integrity, but if persistent senescence leads to excessive scarring and fibrosis. The molecular mechanisms of cellular senescence underlying tissue fibrosis are multifactorial and context-dependent. In this section, recent findings on the reported mechanisms involved in cellular senescence in fibrosis are summarized, using IPF as an example.

Cellular phenotypic changes

Senescent alveolar epithelial type II (AEC2) cells have been implicated in IPF pathogenesis [48,49]. These cells are susceptible to injury and acquire a phenotype showing markers of stress activation as well as senescence [50]. These senescent AEC2 cells undergo repeated alveolar damage and secrete high levels of the SASP, promoting not only cellular senescence of neighboring AEC2 cells but also the accumulation of fibroblasts and myofibroblast transformation, resulting in increased extracellular matrix (ECM) accumulation and disposition. In a recent study, AEC2 cells deficient in insulin-like growth factor binding protein 2 (IGFBP2) led to p21- and phospho-H2AX-mediated

senescence, thereby promoting persistent lung fibrosis [51]. Intriguingly, IGFBP2 expression was significantly lower in AEC2 cells obtained from fibrotic lung lesions of IPF patients compared with patients without IPF. In addition to AEC2 cells, other senescent stromal cell types have been suggested to promote IPF. IPF fibroblasts showed higher expression of β-galactosidase, p21, p16, p53, and the SASP [52]. These cells also had lower proliferation capacity and were resistant to apoptosis compared with age-matched controls. Senescent IPF fibroblasts secrete the SASP, which includes growth factors [fibroblast growth factor (FGF), PDGF, connective tissue growth factor (CTGF)], proinflammatory cytokines [IL-1β, IL-6, IL-8, TGFβ, and tumor necrosis factor (TNF)], chemokines [monocyte chemoattractant protein-1 (MCP-1)], leukotrienes (LTA4, LTB4), and MMPs that further promote the development of lung fibrosis [43,52,53]. Senescent endothelial cells appear to also play a critical role in pulmonary fibrosis, partly by accelerating the endothelial-to-mesenchymal transition via the SASP [54]. Furthermore, senescent endothelial cell conditioned media transferred to healthy lung fibroblasts could induce myofibroblast transition, demonstrating an endothelial cell (EC)-derived SASP-myofibroblast axis.

Telomeres

Telomeres are specific DNA protein structures located at both ends of the chromosomes. Their role is to protect the chromosomes from degradation, assisted by telomerases [telomerase reverse transcriptase (TERT) and telomerase RNA (TERC)], which replenish the telomere cap. During cell division, the telomere shortens, and this mechanism limits cell proliferation by inducing replicative senescence. Significantly shorter telomeres and increased DNA damage were observed in IPF patients compared with age-matched patients with other chronic diseases [55,56]. Mutations in TERT and other telomere-related genes, combined with lifestyle choices such as cigarette use, have been suggested to contribute to the shortened telomeres in IPF [57–59]. In mouse models, uncapping of telomeres in AEC2 cells caused cellular senescence, lung remodeling, and fibrosis [55]. Telomerase deficiency in *Terc* or *Tert* knockout mice led to replicative senescence in pulmonary alveolar stem cells, epithelial impairment, inflammation, premature pulmonary aging, and tissue fibrosis [60].

Mitochondrial dysfunction

Cellular senescence can be elicited by mitochondrial dysfunction [61], which refers to ROS production, diminished respiratory activity, and release of proapoptotic factors. Dysfunctional mitochondria in lung fibroblasts, AEC2 cells, and alveolar macrophages have all been reported in IPF [62–64]. Specifically in lung fibroblasts, the senescent phenotype was attributed to an auto-amplifying loop involving mitochondrial-derived ROS [65]. Loss of AMP-activated protein kinase (AMPK) activity contributed to mitochondrial dysfunction that regulates myofibroblast function in lung fibroblasts, while metformin, which restores AMPK activity, induced mitochondrial biogenesis and promoted fibrosis resolution in lungs [66]. Dysfunctional mitochondria also contribute to the apoptosis-resistant phenotype of senescent cells, including lung myofibroblasts in fibrotic lung disease. The antiapoptotic proteins BCL-2 and BCL-XL were upregulated in senescent myofibroblasts to ensure that these cells survived and avoided death [67], and targeting these senescent cells improved lung fibrosis *in vivo*. Interestingly, it has been shown that mitochondrial dysfunction and the release of mitochondrial DNA reinforces cellular senescence in IPF. The mitochondrial DNA released from IPF fibroblasts drove the activation of senescence, and pharmacological inhibition of cGAS reduced senescence [68]. Moreover, in alveolar epithelial cells from IPF patients' higher senescent cells were found in association with elevated cGAS staining, and pharmacological inhibition of cGAS or siRNA knockdown reduced senescence, suggesting that mitochondrial dysregulation drives senescence and the SASP in both key cell types in IPF [69]. Extracellular vesicles containing miRNAs have also been shown to transmit senescence from epithelial cells isolated from IPF patients [70].

It is also likely that metabolic reprogramming is important in sustaining the myofibroblast phenotype for generation of substrates for ECM deposition. Therapeutic strategies targeting prosurvival mechanisms in cells with **senolytics** are currently being developed, as described later.

Senescence in relation to SSc

Intriguingly, there are many parallels between aging and SSc, as almost all the hallmarks of aging [1] are present in SSc (Figure 1A). These include genomic instability [71], telomere attrition [72], epigenetic alterations [73], loss of **proteostasis** [74], deregulated nutrient-sensing [75], mitochondrial dysfunction [76], cellular senescence [77], and altered intercellular communication [73]. Specifically, in patient-derived skin fibroblasts, increased cellular senescence, as indicated by increased β-galactosidase staining, as well as elevated p21 and p16 levels, was shown in SSc [76,78].

Increased mitochondria-derived ROS may be responsible for the proinflammatory and senescence phenotype in SSc fibroblasts [76]. Direct evidence of the presence of cellular senescence in SSc has also been demonstrated [77]. Significant increases in the markers of senescence, such as $P16^{INK4A}$ as well as vimentin-positive telomere-associated foci (costaining of DNA damage γH2AX and telomeres), were observed in skin biopsies from patients with SSc compared with age-matched controls. In addition, by querying two datasets generated from skin and isolated fibroblasts, significant enrichment of the senescence gene signature was observed in SSc patients compared with healthy controls. This finding was further confirmed at the single-cell level [77]. Among the cell types identified, fibroblasts were among the few that showed the highest enrichment in senescence score. Most importantly, mapping gene expression by spatial transcriptomic analysis showed consistent enriched senescence signatures in SSc skin and correlated strongly with the ECM score. In a recent study, the presence of cellular senescence in SSc skin was further validated as there was a significant correlation between the fibrosis score and the p21/p16 senescence scores in fibroblasts, measured by immunohistochemistry [79]. Similarly, increased senescence effectors and markers were observed in the lungs of patients with IPF [43,80] and SSc-interstitial lung disease (ILD) [81,82], with senescence markers found in both fibroblasts and epithelium. The link between the senescent cells in SSc and ECM in SSc suggest that there is a functional link.

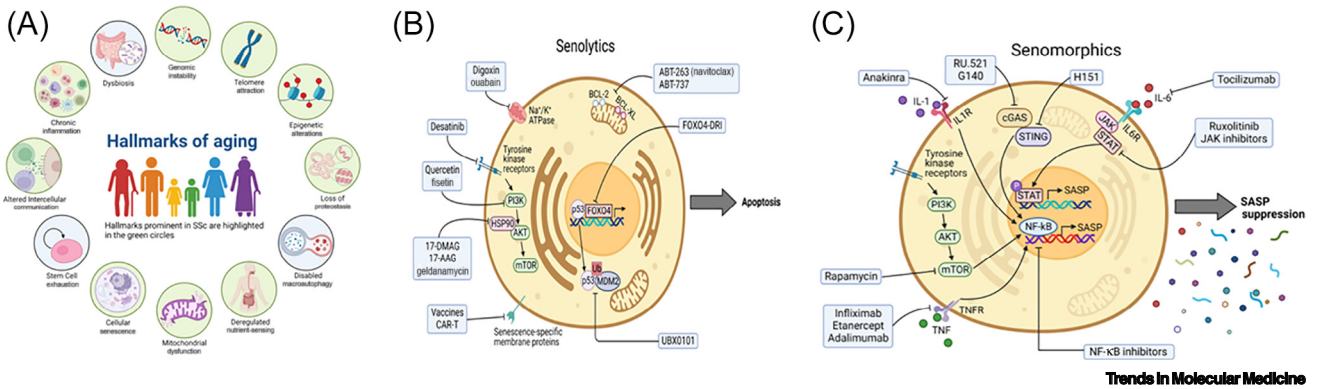

Figure 1. The 12 hallmarks of aging and targeting cellular senescence as an emerging novel therapeutic strategy for fibrosis. (A) Each of the 12 hallmarks of aging [1] represents a different aspect of the aging process. Among these, those that have been found to play significant roles in systemic sclerosis (SSc) are emphasized with green circles. (B) Senotherapeutics could potentially block the profibrotic feedback loop linking tissue injury, autocrine and paracrine cellular senescence, production of the senescence-associated secretory phenotype (SASP), activation of myofibroblasts, and matrix deposition, ultimately resulting in the resolution of fibrosis and restoration of tissue homeostasis. (C) Senotherapeutics currently comprise senolytics and senomorphics. Senolytics are drugs that target molecules or signalling pathways that induce apoptosis in senescent cells. Senomorphics modulate the SASP by interfering with its regulatory pathways or directly neutralize the SASP without affecting cell viability. This figure was created with BioRender. Abbreviations: 17-AAG, tanespimycin; CAR, chimeric antigen receptor; 17-DMAG, alvespimycin; cGAS, cyclic GMP–AMP synthase; HSP90, heat shock protein 90; IL, interleukin; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; STING, stimulator of interferon genes; TNF, tumor necrosis factor; Ub, ubiquitin.

In all, it is evident that cellular senescence is present in SSc. However, the causal role of senescent cells in this disease has yet to be demonstrated. It is possible that senescent stromal cells directly contribute to disease pathogenesis. Senescent fibroblasts exhibit high fibrotic capacity while senescent endothelial cells can promote vasculopathy [77,83]. In addition, senescent cells can secrete the SASP that, in turn, activates neighboring cells in a paracrine fashion, thereby spreading senescence. Moreover, senescent immune cells, which are also highly enriched in the senescent signature in SSc skin [77], could contribute to the pathogenesis of SSc by their defective functions that result in the induction of autoimmunity and inflammation, as well as their impaired ability to eliminate senescent cells. Recently, it was shown that senescent cells are elevated in SSc fibroblasts and, importantly, that transfer of the media from these cells to healthy dermal fibroblasts led to an increase in collagen content [84]. Most interestingly, treatment of senescent dermal fibroblasts with dasatinib and quercetin led to selective loss of the senescent dermal fibroblasts, with reduced collagen content [84]. Precisely what triggers fibrosis via the SASP is not at all clear, with the need for more detailed mechanistic studies warranted. It is likely to be a growth factor and/or cytokine constituents of the SASP. In experimentally induced senescence of normal healthy dermal fibroblasts, the cell media had significantly elevated levels of the growth factor vascular endothelial growth factor (VEGF), suggesting that VEGF may mediate the profibrotic effects. Clearly other factors in the SASP could play a key role in inducing fibrosis, and further research is needed. It has also been demonstrated that M2-type macrophages (widely considered to be profibrotic) could promote mesenchymal cell senescence and that the SASP from the M2 macrophages impaired the ability of stromal cells to attenuate fibroblast to myofibroblast transition [85].

Therapeutic targeting of senescence in fibrosis

There is growing interest in developing therapeutics targeting cellular senescence due to their potential for extending lifespan and delaying the onset of age-related diseases. This is made possible by targeting intracellular senescence-associated pathways as well as extracellular membrane proteins that are elevated specifically in senescent cells. These drugs, termed senotherapeutics, are classified into senolytics and **senomorphics**, and are emerging as novel therapeutics to target fibrosis (Figure 1B).

Senolytics

In senescent cells, distinct antiapoptotic pathways are upregulated for survival and, therefore, can be pharmacologically targeted to promote their death (Figure 1C). The combination of dasatinib and quercetin was first introduced to show the feasibility of targeting cellular senescence by their ability to reduce p21, PAI-2, and BCL-xL [86]. Other senolytics have been reported, including ABT-263 (navitoclax) and ABT-737, which are inhibitors of the antiapoptotic BCL-2 family proteins; cardiac glycosides such as digoxin, Na^+/K^+-ATPase inhibitors; geldanamycin, alvespimycin (17-DMAG), and tanespimycin (17-AAG), which are heat shock protein 90 (HSP90) inhibitors; and FOXO4-DRI and UBX0101, which are p53 modulators. Natural compounds such as fisetin also possess senolytic properties. To date, these compounds have been applied to many preclinical models of fibrosis and have shown promising results (Table 1).

Senomorphics

This class of senotherapeutics refers to drugs that reduce the damaging effects of the SASP independent of inducing cell death in senescent cells. Most senomorphics exert their action by interfering with the signaling or transcriptional regulators of the SASP, such as the JAK/STAT, NF-κB, and mammalian target of rapamycin (mTOR) pathways (Figure 1C). Thus, selective neutralizing or blocking antibodies targeting TNF-α, IL-1β, and IL-6 are considered senomorphic agents. An important consideration when choosing senomorphics for studies is that treatment

Table 1. Senescence in fibrosis *in vivo*

Target organ	Disease model	Senotherapeutic agent(s)	Outcomes	Refs
Cardiac fibrosis	Aged INK-ATTAC and C57BL/6J mice	ABT-263 (navitoclax)	Pharmacological or genetic clearance of senescent cells in mice alleviated myocardial hypertrophy and fibrosis	[93]
	Ischemia reperfusion model in C57BL/6J mice	ABT-263 (navitoclax)	Navitoclax reduced senescence and improved cardiac function by attenuating the profibrotic SASP, reducing scar, and enabling increased angiogenesis	[94]
	Human cardiac fibrosis on a chip model	Quercetin, dasatinib	The senolytic drugs led to an improvement in contractile function, reduced passive tension, and downregulated senescence-related gene expression in the tissue	[95]
Renal fibrosis	Renal artery stenosis in INK-ATTAC mice	Quercetin, dasatinib	Both p16-specific and quercetin–dasatinib improved renal function and structure	[96]
	C57BL/6J mice on high fat diet	Quercetin	Improved renal function indices and alleviated fibrosis	[97]
	Aged and/or kidney injury models in C57BL/6J mice	ABT-263 (navitoclax)	ABT-263 treatment resulted in improved functional recovery and reduced fibrosis	[44]
	Lupus nephritis in MRL/lpr mice	Fisetin	Fisetin treatment attenuated kidney fibrosis, reduced SASP expression, and increased tubular epithelial cell proliferation	[98]
Lung fibrosis	Bleomycin lung fibrosis in INK-ATTAC mice	Quercetin, dasatinib	Both p16-specific and quercetin–dasatinib improved renal function and structure	[43]
	Ex vivo fibrotic 3D lung tissue	Quercetin, dasatinib	Pharmacological treatment significantly eliminated senescent markers and the SASP	[99]
	Bleomycin lung fibrosis in C57BL/6J mice	Nintedanib	Nintedanib suppressed senescent cell survival through the JAK2/STAT3 signaling pathway in the lung fibrosis model	[100]
	Intratracheal γ-irradiated IMR90 cells in mice	Digoxin	Digoxin effectively eliminated senescence-induced lung fibrosis	[101]
	Bleomycin and crystalline silica-induced lung fibrosis in mice	ABT-263 (navitoclax)	ABT-263 induced fibroblast apoptosis, decreased fibroblast numbers, and reduced fibrosis	[67,102]
	Bleomycin-induced lung fibrosis in mice	Rapamycin	Rapamycin inhibited pulmonary fibrosis and epithelial–mesenchymal transition in mice	[103]
	Radiation-induced lung fibrosis in C57BL/6 mice	FOXO4-DRI	FOXO4-DRI alleviated pulmonary fibrosis by targeting senescence-like fibroblasts *in vivo*	[104]
	Senescent IMR90 cells-induced lung fibrosis in mice	ABT-263 (navitoclax)	Navitoclax significantly reduced collagen content in fibrotic lungs, comparable with the effect of nintedanib and pirfenidone	[105]
Liver fibrosis	CCl_4- or NASH-induced liver fibrosis in C57BL/6N mice	uPAR-specific CAR T cells	CAR T cells efficiently eliminated senescent cells, reduced fibrosis, and improved liver function in mice	[88]

with these agents is likely to require continuous dosing, as opposed to senolytic drugs, which only require intermittent dosing.

An emerging strategy is to use immunotherapy to specifically kill senescent cells by targeting specific surface markers exclusively expressed by these cells. Senescent cells are removed by immune cells under normal physiological conditions [87]. Aging is associated with a decline in immunosurveillance that allows senescent cells to evade immune clearance. Additionally, senescent cells may also develop immune suppression mechanisms to increase their survival. The development of immunotherapies to target cellular senescence takes advantage of senescence-specific surface markers. These include urokinase-type plasminogen activator receptor (uPAR), glycoprotein nonmetastatic melanoma protein B (GPNMB), dipeptidyl peptidase 4 (DPP4), and NOTCH receptors. A recent study [88] explored the use of chimeric antigen receptor (CAR) T cells to target senescent cells. The results of this study showed that uPAR-specific CAR T cells efficiently eliminated senescent cells both *in vitro* and *in vivo*. In addition, the CAR T cells also extended the survival of mice with lung adenocarcinoma, and effectively eliminated senescent cells, reduced fibrosis, and improved liver function in mouse models of CCl_4- or nonalcoholic steatohepatitis (NASH)-induced liver fibrosis.

Clinical evidence of senescence targeting

The aforementioned studies clearly demonstrate that the accumulation of senescent cells is critically involved in fibrosis, and pharmacologically clearing them or removing the SASP can alleviate fibrosis in various preclinical models. These studies have laid the foundation for testing senotherapeutics in human clinical trials. Although there are more than 20 ongoing or completed clinical trials of senotherapeutic therapies focusing on various conditions, there are only three trials that have been designed to test the effectiveness of senolytics in fibrosis (Table 2). One recently completed trial for IPF is a confirmatory, randomized, placebo-controlled trial of dasatinib and quercetin in patients with IPF to evaluate the feasibility and tolerability of the drugs relative to a placebo [89]. The participants self-administered either placebo or intermittent dasatinib (100 mg orally per day) and quercetin (1250 mg orally per day), taken over 3 consecutive days in 3 consecutive weeks, resulting in nine doses in total. This dosing regimen was shown to be feasible and well-tolerated in the patients, with a roughly threefold increase in nonserious adverse events and a modest increase in limiting symptoms in the dasatinib–quercetin arm. The multicenter, single-arm, open-label Phase 2a trial of dasatinib in SSc-ILD was designed to test the effectiveness of blocking tyrosine kinases (targeting c-Abl, PDGF receptor, and Src kinase) to treat SSc fibrosis [90] (see Clinician's corner). While dasatinib was well-tolerated in patients with early-stage ILD, there were no significant changes in the **modified Rodnan skin score** (**MRSS**) or pulmonary function tests (PFTs). Skin biopsy-based gene expression analyses revealed that patients mapping

Clinicians' corner

The detection and measurement of cellular senescence in various tissues remains challenging, and, to date, there are no universal sensitive and specific markers.

Although cellular senescence might play homeostatic, regenerative, and protective roles, increasing evidence indicates that excessive, persistent, or premature accumulation of senescent cells in tissues such as the lungs, heart, articular cartilage, and skin contributes to the failure of these organs.

Mitigating the deleterious impact of senescent cells using genetic and pharmacological strategies has been associated with amelioration of diverse pathological states in preclinical models. Potential therapeutic approaches include senolytic agents (senescence clearance), senomorphic drugs, and immunotherapies such as CAR T cells to target and eliminate senescent cells. The field of 'senotherapeutics' boasts a rich pipeline of agents to be tested in preclinical studies and clinical trials.

To date, the optimal antisenescence approach in disease treatment is unknown, and the safety and clinical efficacy of such interventions remains to be demonstrated. Similarly, the clinical indications best poised to benefit from antisenescence therapy require further investigation. Nonetheless, senotherapeutics offers an exciting new avenue for the treatment of fibrosis and age-related human pathologies.

Table 2. Clinical trials targeting fibrotic conditions with senotherapeutics

Indication	Study ID	Drug regimen	Status	Refs
Idiopathic pulmonary fibrosis	NCT02874989[ii]	Dasatinib, quercetin	Completed. The dosing regimen is feasible and well-tolerated in these patients	[89,106]
SSc pulmonary fibrosis	NCT00764309[iii]	Dasatinib	Completed. Treatment was associated with an acceptable safety profile but no significant clinical efficacy	[90]
Nonalcoholic fatty liver disease with fibrosis	NCT05506488[i]	Dasatinib, quercetin	Currently recruiting	–

into the inflammatory gene expression subset were mostly non-improvers. Reanalysis of the trial results revealed that the gene signature of the SASP decreased significantly from the baseline in dasatinib-treated improvers, while no difference from the baseline was found in non-improvers [91]. A double-blind randomized controlled proof-of-principle study to test dasatinib–quercetin in nonalcoholic fatty liver disease is currently ongoing (NCT05506488[i]).

Concluding remarks

Cellular senescence is now recognized as a fundamental hallmark of aging. Senescence indeed is the driver of aging-associated multimorbidity [92]. While transient accumulation of senescent cells plays an important role in development and in adult tissue homeostasis, persistent accumulation of senescent cells, due to their increased emergence and/or defective elimination, drives inflammation, fibrosis, and other pathologies during aging and in multiple diseases (Figure 2). The molecular and metabolic reprogramming that occurs as normal cells transform into senescent cells is becoming increasingly understood. Many of these insights have been obtained from animal studies, and their relevance to human aging and diseases remains to be established. Further studies are needed to confirm the pathogenic role of cellular senescence in SSc, and to uncover the mechanistic bases. Identifying the cell lineages showing senescence, and their contributions to specific clinical and pathological disease manifestations in SSc will be important. For instance, whether it is fibroblast senescence, endothelial cell senescence, immune senescence, or a combination of all of these that accounts for the stage-specific manifestations and morbidity of SSc remains obscure.

Outstanding questions

What are the upstream drivers of senescence in tissue fibrosis?

What is the precise role of the SASP in mediating fibrosis?

Would selective elimination of senescent cells in tissue fibrosis in humans retard already established fibrosis?

Would selective elimination of senescent cells increase cancer risk?

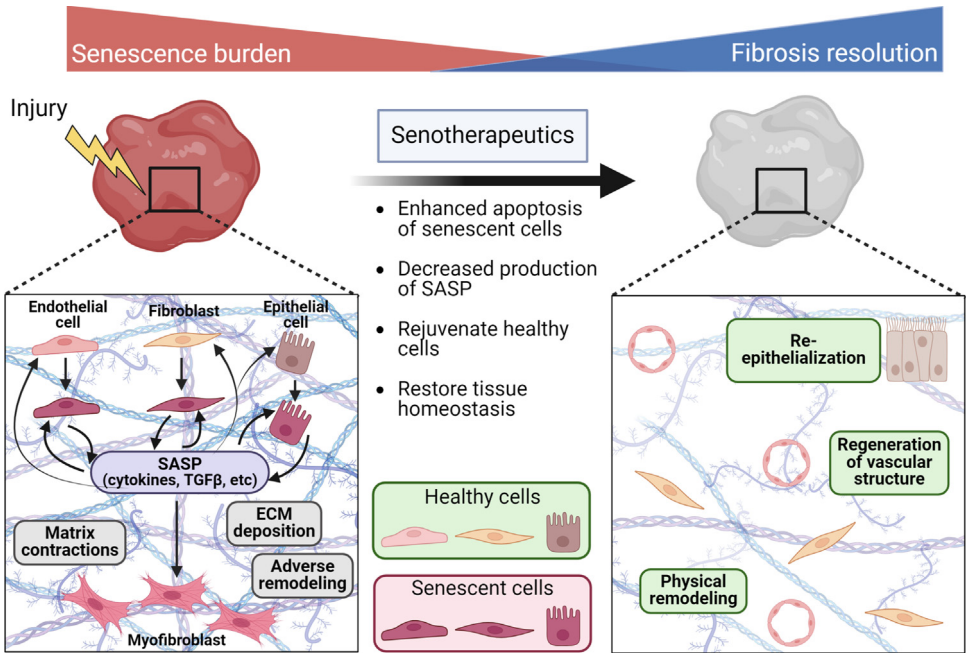

Figure 2. Possible role of senescent cells in mediating fibrosis. An upstream activator such as injury may promote tissue damage and a senescent response could ensure the restoration of normal tissue integrity and homeostasis. If this is not terminated appropriately through the removal of senescent cells, then fibrosis within the organ can occur. Feedforward loops between senescent and non-senescent cells amplify and reinforce senescence. The senescence-associated secretory phenotype (SASP) released by senescent cells may be the driving force in this via factors [cytokines, transforming growth factor beta (TGFβ), etc.] that act in a paracrine fashion leading to increased deposition of extracellular matrix (ECM) and fibrosis, and most likely a combination of SASP constituents. Resolution of fibrosis may occur through the selective elimination of senescent cells and reduced SASP, leading to restoration of normal tissue architecture and could be promoted by senotherapeutic agents. This figure was created with BioRender.

Nevertheless, recent observations emerging from the senescence field have sparked broad interest in therapeutic targeting of cellular senescence for many conditions currently considered to be incurable. Potential senotherapeutic approaches include senolysis, the selective removal of senescent cells, which has shown potential efficacy in preclinical disease models of fibrosis, as detailed earlier. Senolytic treatment is likely to deploy drug repurposing, and offers the advantage of allowing intermittent pulse therapy, thus mitigating potential toxicity. However, the selectivity of such agents for eliminating only senescent cells and only those lineages that have been shown to directly contribute to the pathogenesis of disease remains a difficult challenge. Other approaches include senomorphics, which modulate specific signaling pathways without killing senescent cells, and senopreventive agents. While results from early clinical trials offer encouragement, much work remains before we are ready to embrace senotherapeutics for the treatment of SSc or fibrosis (see Outstanding questions).

Further mechanistic insights into how precisely senescent cells and, in particular, the factors contained within the SASP promulgate fibrosis will help define new targets for therapeutic gain. It is likely that more senomorphs will be discovered and repurposed from other indications, and these should be tested in fibrotic disease.

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Declaration of interests

The authors declare no conflicts of interest.

Resources

[i]https://clinicaltrials.gov/study/NCT05506488

[ii]https://clinicaltrials.gov/study/NCT02874989

[iii]https://clinicaltrials.gov/study/NCT00764309

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